© 2019. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

RESEARCH ARTICLE

Tackling the Tibetan Plateau in a down suit: insights into thermoregulation by bar-headed geese during migration

Nicole Parr[1], Charles M. Bishop[2,*], Nyambayar Batbayar[3], Patrick J. Butler[4], Beverly Chua[5], William K. Milsom[5], Graham R. Scott[6] and Lucy A. Hawkes[7]

ABSTRACT

Birds migrating through extreme environments can experience a range of challenges while meeting the demands of flight, including highly variable ambient temperatures, humidity and oxygen levels. However, there has been limited research into avian thermoregulation during migration in extreme environments. This study aimed to investigate the effect of flight performance and high altitude on body temperature (T_b) of free-flying bar-headed geese (*Anser indicus*), a species that completes a high-altitude trans-Himalayan migration through very cold, hypoxic environments. We measured abdominal T_b, along with altitude (via changes in barometric pressure), heart rate and body acceleration of bar-headed geese during their migration across the Tibetan Plateau. Bar-headed geese vary the circadian rhythm of T_b in response to migration, with peak daily T_b during daytime hours outside of migration but early in the morning or overnight during migration, reflecting changes in body acceleration. However, during flight, changes in T_b were not consistent with changes in flight performance (as measured by heart rate or rate of ascent) or altitude. Overall, our results suggest that bar-headed geese are able to thermoregulate during high-altitude migration, maintaining T_b within a relatively narrow range despite appreciable variation in flight intensity and environmental conditions.

KEY WORDS: *Anser indicus*, Avian flight, Biologging, Body temperature, High altitude

INTRODUCTION

Every year, billions of animals make long-distance migratory journeys between productive winter foraging grounds and seasonably suitable breeding grounds (Hahn et al., 2009; Hu et al., 2016; Somveille et al., 2015). Migrating birds can be found travelling through a range of ambient conditions, exposing them to extremes of temperature, water and oxygen availability (Adamík et al., 2016; Tombre et al., 2008). Birds that migrate at high altitude may experience all of these: extreme cold, low humidity and reduced oxygen availability as a result of declining air density (Altshuler and Dudley, 2006; Butler, 2010). Hypoxia imposes severe challenges on the ability of birds to cope with cold temperatures, especially at high altitude, because thermogenesis is costly in terms of oxygen consumption (Bicudo et al., 2002; Dawson and Carey, 1976; Withers, 1977). Whilst in some waterfowl the energetic cost of thermoregulation is considered irrelevant in calculations of daily energy expenditure (given the relatively greater costs of locomotion, feeding, courtship and vigilance; McKinney and McWilliams, 2005), in extreme environments where ambient temperatures may fall below the thermoneutral zone, metabolic costs to maintain thermal neutrality contribute a larger proportion of daily energy expenditure, especially during migratory stopovers (Williams et al., 2014). Little previous work, however, has examined avian thermoregulation during migration in extreme environments.

Bar-headed geese (*Anser indicus*) migrate annually from warm wintering grounds in south Asia to breeding grounds in Mongolia (Hawkes et al., 2011), crossing the Himalayas and Tibetan plateau (approximately 1000 km north to south with a mean elevation of 4500 m). The Tibetan Plateau can be inhospitable to migrants, with minimum monthly temperatures below −30°C for September to November (Ding et al., 2018), when bar-headed geese complete their southward migration. How these birds are able to thermoregulate in extreme cold both at stopover sites and during flight has not been investigated.

Resting birds experiencing cold ambient environments, and thus increased thermoregulatory demands, can decrease core body temperature by 2–3°C overnight, accompanied by reduced activity levels (Geiser, 2004; Welton et al., 2002), conserving energy that would otherwise be spent on thermogenesis during overwintering periods. This response is most common in small species (Clarke and Rothery, 2008), such as hummingbirds (*Trochilidae*), with high specific energy requirements, low thermal inertia and narrow zones of thermal neutrality (Hiebert, 1990). Overnight reductions in body temperature (T_b) cause large ranges of circadian T_b cycles, with minimum T_b below normothermic levels (McKechnie and Lovegrove, 2002) and a reduced response to external stimuli (Carpenter and Hixon, 1988). Given the larger body size of waterfowl, behavioural modifications such as huddling or changes in posture to reduce exposure of non-feathered areas (Brodsky and Weatherhead, 1984) may be sufficient to sustain T_b within normothermic levels during periods of relative inactivity, such as at staging areas. However, during periods of migration, bar-headed geese usually begin flights at night or early in the morning (i.e. in the coldest part of the day) (Hawkes et al., 2012). This suggests bar-headed geese do not conserve energy through suppression of T_b overnight during migration (which may impair flight performance; Carr and Lima, 2013). Furthermore, bar-headed geese may not use behavioural changes such as huddling or positioning to retain heat during the coldest periods, but perhaps generate sufficient heat through flight activity (exercise thermogenesis) to sustain a

[1]University of Exeter, Centre for Ecology and Conservation, Penryn Campus, TR10 9FE, UK. [2]Bangor University, School of Biological Sciences, Bangor LL57 2UW, UK. [3]Wildlife Science & Conservation Centre of Mongolia, Bayanzurkh District, Ulaanbaatar, 210351, Mongolia. [4]School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. [5]Department of Zoology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4. [6]Department of Biology, McMaster University, Hamilton, ON, Canada, L8S 4K1. [7]University of Exeter, College of Life and Environmental Sciences, Hatherly Laboratories, Exeter EX4 4PS, UK.

*Author for correspondence (c.bishop@bangor.ac.uk)

N.P., 0000-0001-6115-6816; C.M.B., 0000-0002-9030-4344; L.A.H., 0000-0002-6696-1862

Received 21 March 2019; Accepted 17 September 2019

Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

consistent T_b. By flying in cold conditions, geese complete flights in air with a higher density than would be experienced at similar altitudes later in the day (when it would be warmer), thus requiring less metabolic power to fuel flight (Pennycuick, 1969), and may reduce thermoregulatory costs by resting during the day. Because bar-headed geese forage diurnally on vegetation in wintering and breeding grounds, we hypothesise firstly that during the non-migratory period, there will be a diurnal signal in core T_b, with peak T_b occurring in the middle of the day. However, during the migratory period, when they may spend more time at altitude in cold conditions, we hypothesise that this will shift to reach peak T_b overnight, even on days with no flight activity.

In addition to the potential energy savings through overnight reductions in T_b described in some species whilst at rest or overwintering (Bicudo et al., 2002), it has been suggested that maintaining a lower T_b during or prior to migration (rather than only at night) reduces thermogenic costs and allows birds to better conserve fat stores (Carpenter and Hixon, 1988). This has been demonstrated in species of waterfowl such as barnacle geese (*Branta leucopsis*), which decrease mean T_b by up to 4.4°C in the first 20 days of migration (Butler and Woakes, 2001). This energy conservation may serve to reduce the depletion of fat stores during migration, given the lower thermogenic requirements. It remains to be seen whether bar-headed geese also exhibit a decrease in T_b before or during migration, which, although beneficial for energy conservation, may lead to impaired metabolic function (Carr and Lima, 2013).

In contrast to the risk of hypothermia presented by high-altitude environments, the energetic cost of flight at sea level may lead to hyperthermia, which may limit flight duration (Bevan et al., 1997; Butler et al., 1977). There are a number of physiological maladjustments that excessive heat gain might cause, including damage to enzymes, increased oxidative stress and dehydration (Dawson, 1982; McKechnie and Wolf, 2019). Sufficient heat can be generated during flight to reduce flight duration to 15 min in migrating eider ducks (*Somateria mollissima*), a predominantly diving species (Guillemette et al., 2016), and necessitate periods of cooling following these flights (Guillemette et al., 2017). Bar-headed geese can complete long flights of up to 8 h (Hawkes et al., 2011), suggesting they may not be constrained in such a way, nor spend large periods of time cooling after flight. During steep climbing flights from Mongolia onto the Tibetan Plateau, bar-headed geese do have concurrent increases in T_b (Bishop et al., 2015), but it is not known whether this limits flight activity, nor whether this generates heat that can be stored following flight. Thus, how closely flight effort and T_b are related in bar-headed geese, and whether they modulate flight effort to ascend over mountainous areas whilst avoiding potential hyperthermia during flights or hypothermia whilst at rest is unknown.

Specific aims

Biologging techniques provide the opportunity to better understand the physiological challenges that extreme environments may present to animals, and how these challenges are met (Bishop et al., 2015; Ciancio et al., 2016). The present study aimed to (i) describe bar-headed goose T_b before, after and during the trans-Himalayan and Tibetan Plateau migration, and investigate whether (ii) bar-headed geese display a strong diurnal signal in core T_b that is disrupted by the timing of their migratory flights, (iii) T_b decreases at the onset of migration and (iv) varying flight effort and altitude cause changes in T_b.

MATERIALS AND METHODS

Tagging and retrieval

Bar-headed geese, *Anser indicus* (Latham 1790), were captured during their annual primary flight feather moult at Terkhiin Tsagaan Lake, Mongolia (48.148°N, 99.577°E) in July 2010 and July 2011, using 'corral trapping' (Whitworth et al., 2007). Custom-built loggers (Spivey and Bishop, 2014) were surgically implanted into the abdominal cavity of 59 geese (29 were implanted in 2010 and a further 30 in 2011) under isoflurane anaesthesia; the loggers recorded heart rate at 180 Hz, acceleration in three axes (surge, heave and sway at 100 Hz, in 18 s bursts every 2 min) and intra-abdominal temperature and pressure (once every 30 s) for up to 222 days. We considered intra-abdominal temperature to be analogous to core T_b. Loggers weighed 32 g (1.5% of the smallest bird's body mass), which is well below the recommended maximum 3% body mass for tracking devices (Kenward, 2000). Given the potential for a negative impact of surgery on the birds, it was not considered appropriate to co-deploy loggers with tags that geolocated the birds or sampled the environmental conditions as they flew. Pressure (measurement range 1–110 kPa, ±10 Pa) was converted into estimates of altitude in the international standard atmosphere using the sensor manufacturer conversion of $44331\times[1-(1.01325\times\text{pressure in mbar}/1000)^{0.1902}]$ and is thus referred to as 'altitude' hereon, accepting that daily variations in temperature affect the altitudinal estimates. Geese were administered a long-lasting analgesic (buprenorphine) and released at least 4 h after surgery when they were awake, alert and in superficially good health, on the same day as capture. Geese were not kept in captivity overnight following surgery as we deemed this an additional stressor rather than likely to lead to better recovery. We observed no mortality following logger implantation and birds returned to their flocks following release. The following year, geese were recaptured at the same location to recover the loggers (10 geese were recaptured in 2011 and 16 in 2012).

Of the 26 recovered loggers, seven provided datasets [referred to as geese A–G, including five geese (geese C–G) that provided data presented in Bishop et al. (2015), where they were referred to as 35, 37, 38, 41 and 43, respectively] that described T_b during the southward migration (3 female, mean±s.d. body mass 2.323±0.184 kg; 3 male, 2.523±0.092 kg; and one goose that was not sexed, 2.67 kg), of which four datasets contained heart rate, acceleration, T_b and altitude data, but three failed to record heart rate. Only five of these loggers included the complete southward migration, as the loggers failed in two geese (A and B) before the migration was completed (Fig. S1). The loggers of 19 birds failed to record T_b and are therefore not included in the present study. Collecting such high-resolution datasets from wild birds in extreme environments is clearly challenging and led to a relatively small sample size and we note that it is possible that the birds that loggers were recovered from may have been atypical. After departing their breeding grounds in Mongolia, all geese flew southwards onto the Tibetan Plateau, which extends as far as the northernmost part of Nepal and Bhutan, crossing the Himalayan Mountain range at its southern border shown in the altitude signal (Fig. 1A). Four geese then continued to low-altitude wintering grounds; one goose (G) did not make this descent and over-wintered between 3000 and 4000 m.

Data processing

First, every 2 min throughout the logger data collection period, data were summarised into mean values for heart rate (raw ECG summarised to beats min^{-1} using a custom script in R; Spivey and Bishop, 2014), T_b and altitude. Using the date of deployment and sampling frequency, we added a date and time stamp to each 2 min

Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

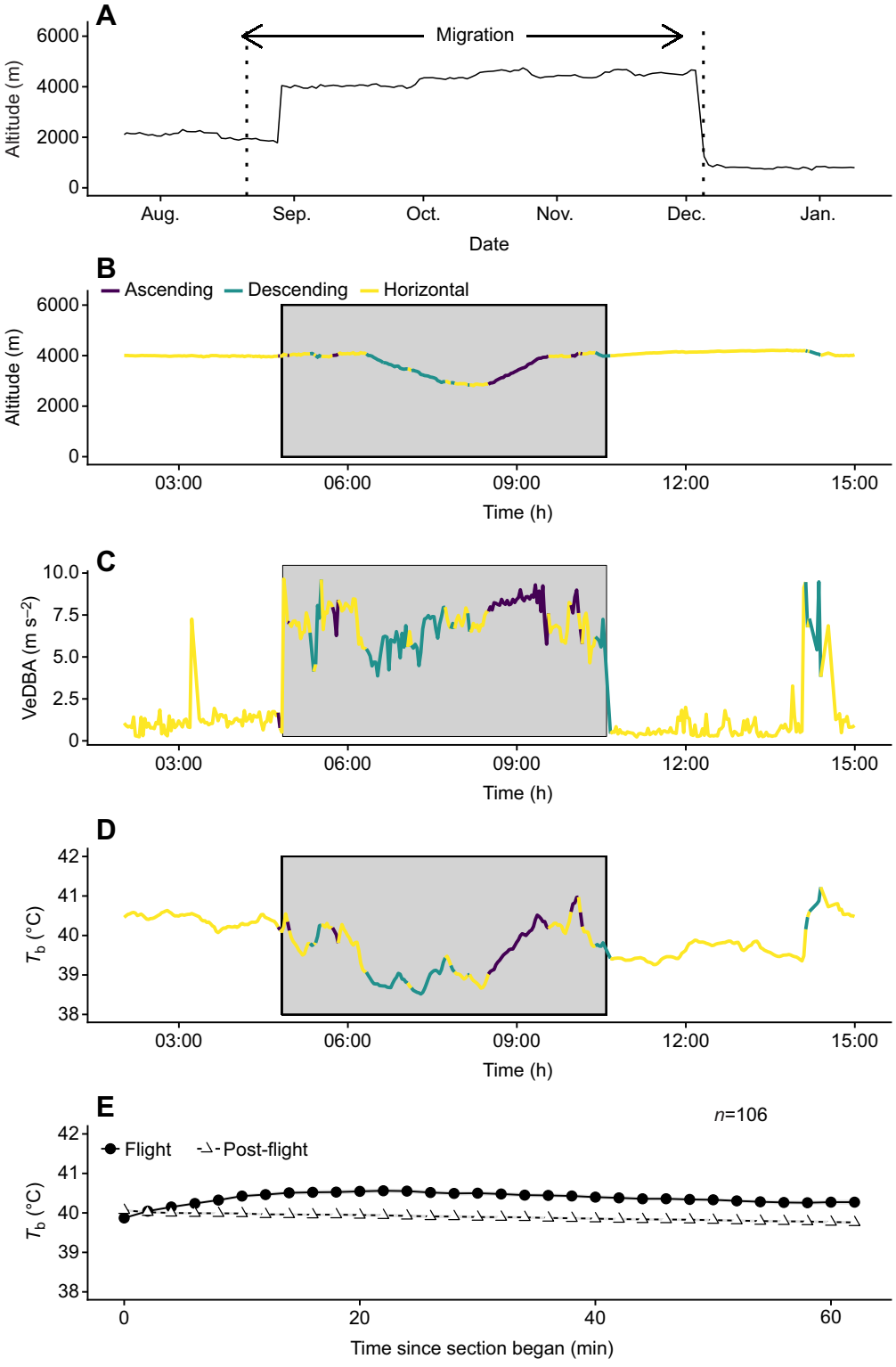

Fig. 1. Representative data collected during migration. (A) The altitude experienced (*y*-axis) during the complete tracking period (*x*-axis) for one goose (goose D); dotted vertical lines delineate the migratory period. (B–D) A 12 h window of data for goose D (time on *x*-axis), including an example of a long migratory flight (grey shaded box) of 340 min on 6 September 2011. Data are shown for (B) altitude, coloured to reflect rates of descent greater than −0.1 m s^{-1} (green), rates of ascent greater than +0.1 m s^{-1} (purple) and horizontal flight (yellow) with rates of descent/ascent between −0.1 and +0.1 m s^{-1} (colouring also applies to C and D); (C) acceleration data (vectoral dynamic body acceleration, VeDBA); and (D) concurrent variation in body temperature (T_b) between 38.5 and 41.2°C. (E) Mean T_b during the first hour of long flights (black circles) and the first hour following flight (white triangles) of all long migratory flights across all geese (*N*=7), where geese rested for at least 1 h after the flight (*n*=106 flights).

block (Fig. 1A–D). The tri-axial acceleration data (surge, heave and sway) were processed to derive two metrics for each 2 min block: (i) root mean square dorsoventral acceleration ($\ddot{Z}_{rms}$, m s^{-2}) and (ii) vectoral dynamic body acceleration for total body movement (VeDBA, m s^{-2}; Qasem et al., 2012; Fig. 1C). The rate of change of T_b (°C min^{-1}) and the rate of change in altitude (referred to as rate of ascent/descent, m s^{-1}) were calculated and appended to the dataset.

The distribution of $\ddot{Z}_{rms}$ data was plotted, which yielded two clear groups of $\ddot{Z}_{rms}$ above and below 2 m s^{-2} (Fig. S2), which were considered to represent stationary and flight periods, respectively. Individual flights were then identified as continuous sections of $\ddot{Z}_{rms}$ above 2 m s^{-2} that lasted at least 6 min, and assigned unique IDs. This threshold yielded 2121 flights between 6 min and 17.4 h, by seven geese (Table 1).

Journal of Experimental Biology

Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

Table 1. Summary of the number of long and short flights per goose and the mean, minimum and maximum goose T_b during the migration period, while stationary and flying

	Migration date		No. of flights (% migration time)		Mean T_b (range) (°C)	
Goose	Start	Finish	<60 min	>60 min	Flight	Stationary
A	16/08/2011	02/10/2011*	218 (1.2)	42 (5.8)	40.425 (38.61–42.12)	39.886 (38.26–41.88)
B	08/08/2011	30/09/2011*	142 (0.8)	12 (2.7)	39.793 (37.02–42.73)	39.624 (36.87–41.29)
C	23/08/2011	29/11/2011	102 (0.4)	12 (3.3)	40.117 (37.79–42.29)	39.629 (37.49–41.79)
D	21/08/2011	04/12/2011	582 (0.6)	108 (6.3)	40.45 (37.66–42.17)	39.813 (37.59–41.81)
E	10/08/2011	17/11/2011	444 (0.6)	68 (4.5)	40.107 (36.89–41.82)	39.717 (36.48–42.13)
F	12/09/2011	26/11/2011	205 (0.6)	26 (3.0)	40.037 (38.04–41.56)	39.722 (37.44–41.74)
G	25/08/2011	04/11/2011	147 (0.7)	13 (2.3)	40.722 (38.82–43.51)	39.994 (38.35–42.0)

Dates are given as day/month/year. Asterisks indicate the date loggers failed on rather than the end point of migration. T_b, body temperature.

Although the geese were not simultaneously tracked with geo-loggers, it was possible to make a coarse estimate of their location using altitude data to detect periods at the breeding grounds, crossing the Himalayas and the Tibetan Plateau (the mean elevation of Mongolia, approximately 2000 m, is more than a 1000 m lower than the flyway across the Tibetan Plateau at approximately 4500 m) and at low-altitude wintering grounds in India (at less than 1000 m). We deemed the timing of the climb onto the Tibetan Plateau from Mongolia in the north in combination with the onset of long (>60 min) flights as the onset of migration, and the descent into wintering grounds in combination with flight duration as the end of migration (Fig. 1A; Fig. S1). Therefore, hereon in we refer to this period as migration, accepting that some birds may have continued flying through India; however, without the ability to geolocate the birds, we consider this approach to be the most parsimonious.

Statistical analysis

All statistical analysis was carried out in R (http://www.R-project.org/). Whenever sample size was sufficient (N>5 geese) we used mixed models with REML to analyse trends in T_b with flight ID nested inside goose ID (all models fitted using the nlme package (https://CRAN.R-project.org/package=nlme). When analysis looked for trends within flights, we only included long flights (>60 min) to provide a sufficient number of data points. Differences between levels within one factor (i.e. stage of year included before, during and after migration) were compared with Tukey's *post hoc* pairwise comparison and stated as significant when P<0.05 (following Williams et al., 2019). We calculated the amount of variance explained by the random factors or fixed effects using marginal R^2 values (Nakagawa and Schielzeth, 2013) and extracted the variance components from each of the models. Given the highly autocorrelated nature of biologging datasets, we included a corAR1 correlation term in all mixed models. Following Harrison et al. (2017), when the number of geese for a given analysis fell below a threshold of five, we generated models and performed statistical tests for each goose separately, with statistical results reported in the supplementary information (Tables S1 and S2). Residuals of all models were checked to confirm model assumptions, including normality and homoscedasticity. Because of the small sample size, in addition to reporting results across all geese we also report individual trends amongst geese and have used figures to highlight differences between geese. We used the following analyses to address each of the following questions in turn: (1) Is circadian rhythm altered during migration? (2) Does T_b vary before, during and after migration? (3) Does T_b change during flight? (4) Does T_b change with altitude?

Seasonal patterns in T_b

Is circadian rhythm altered during migration?

To determine whether daily patterns of T_b differed between migratory and non-migratory periods, we first corrected time stamps from GMT to local time, and calculated the median time (hour of day) that the peak daily T_b occurred during migratory and non-migratory periods using circular statistics (following Jammalamadaka and Lund, 2006), and then tested whether these times were uniformly distributed (Rayleigh's test of uniformity). We then used a Watson–William's test to assess whether the temporal distribution of peak T_b differed between migratory and non-migratory periods. This was repeated on data filtered to include only days when no migratory flights occurred. In order to remove the temporal autocorrelation, we randomly discarded 33% of the data (which was sufficient to prevent a given day being a significant predictor of the following day, as determined by inspection of auto-correlation function plots; Fig. S3). Finally, we used a mixed model (with goose ID as a random intercept and slope) to test whether the daily range in T_b differed outside of migration from that during migration, with a corAR1 correlation term.

Does T_b vary before, during and after migration?

To assess whether there were changes in T_b relating to the onset of migration, we calculated a mean daily temperature for each goose excluding periods of flight. We then used a mixed model with stage of year relative to migration (pre-, during or post-migration) as a fixed effect and day nested inside goose ID as a random intercept and slope to test for differences between mean daily temperature relative to migration, with day included as a corAR1 correlation term to control for temporal autocorrelation.

Changes in T_b with flight effort and altitude

Does T_b change during flight?

To investigate whether flight activity increased T_b, we first calculated the change in T_b (ΔT_b) for each long flight (>60 min) in the migratory period. We then used a linear mixed model to test whether T_b differed at the start or end of a flight with time of measurement (either start or end of flight) as a fixed effect to predict T_b. Individual flight ID was nested inside goose ID as a random intercept, and flight duration was included as a corAR1 correlation term in order to account for temporal autocorrelation amongst varying durations of flight. We also investigated the number of flights that stopped at a peak T_b for that flight and describe the pattern of heat gain and loss within a flight cycle.

Does T_b change with altitude?

To assess whether T_b changed systematically with altitude, we used a mixed model with flight status (stationary or in-flight), altitude

(binned into 1000 m bins) and the interaction between flight and altitude as fixed effects. We nested flight (or stationary bout) ID inside goose ID as a random intercept and included a corAR1 correlation term to account for temporal autocorrelation. To investigate the effect size of altitude and flight status on goose T_b we calculated 95% confidence intervals (CI) for each coefficient. To better explore the potential drivers of changes in T_b while geese were in flight, heart rate, rate of ascent and altitude were used as fixed effects to predict T_b. We included all data recorded during flights over 60 min to detect shifts within flights. Because of the small sample size in this analysis (only four geese provided heart rate data), we modelled each goose separately with flight ID included as a random intercept. Given the high temporal autocorrelation present in the data, we again included a corAR1 correlation term in the model. To account for the non-linearity in the predictor's relationship with T_b, we binned each of the predictors; heart rate was grouped into 50 beats min^{-1} bins, rate of ascent/descent was grouped to 25 m s^{-1} bins and altitude was grouped into 1000 m bins.

RESULTS

Flight and T_b metrics

The seven geese provided a total of 281 long (>60 min) migratory flights (representing 626 h of flight, mean 2.29 h per flight, range 1–17 h) and a total of 1840 short (6–59 min) flights throughout the tracking period (representing 613 h of flight, mean 20 min per flight; Table 1). Long migratory flights occurred throughout the migration south after breeding (Table 1; Fig. S1), with geese flying for 4.9 h a day (mean across geese, 3.4–7.2 h) interspersed with days of no long flights, completing migratory flights on a mean of 21 flight days (goose G differed from the other geese and completed long flights on only 7 days, instead using shorter flights than the other geese, and over-wintered at a higher altitude than the other geese; Fig. S1). Goose D flew on the largest number of days, completing long migratory flights on each of 48 days (Table 1; Fig. S1) interspersed with days of no migration. The loggers of geese A and B failed before the geese completed their migration (Table 1; Fig. S1).

Throughout the tracking period, grand mean (±s.d.) T_b was 39.89±0.14°C. Daily T_b ranged by 2.37±0.17°C (mean±s.d. across geese). The mean maximum T_b recorded across all geese was 42.38°C (range across geese 42.29–43.51°C), the mean minimum T_b measured was 37.48°C (range 36.48–37.59°C; Table 1). The rate of change of T_b was relatively constant during flight (mean±s.d. 0.02±0.017°C min^{-1}) and was slower when stationary (−0.002±0.0005°C min^{-1}) (Fig. 1E).

Seasonal patterns in T_b

Is circadian rhythm altered during migration?

Geese reached their highest T_b per day earlier during migration than during non-migratory periods [02:32 h (range 00:13 h–07:36 h) versus 11:53 h (07:05 h–16:13 h), circular median time across all seven geese; Watson–Williams test, $P \leq 0.01$ in all geese; Fig. 2; Table S1, Fig. S4]. The temporal shift was reflected in activity patterns as well, with geese becoming more active at approximately 20:00 h and decreasing activity at approximately 12:00 h the next day, remaining active all night. Time of peak T_b per day was non-uniformly distributed in all geese (Rayleigh's test, $P<0.05$ in all geese, range <0.001 to 0.04; Table S1); geese reached peak T_b between 00:30 h and 03:00 h during migration, with the exception of goose C (median time of peak T_b 07:36 h). During non-migratory periods, geese displayed a diurnal pattern in T_b (Rayleigh's test of uniformity, $P<0.05$ in all geese, range <0.001 to 0.037; Table S1), becoming active and warmer at approximately 04:30 h to 05:00 h and settling down and becoming cooler at 20:00 h, with T_b ranging over 2.64°C day^{-1} (mean daily value; Fig. 2). During days with no migratory flights, we observed the same trend for earlier peak T_b relative to that outside of migration (though this was not significant in goose C; Table S1). Variation in T_b between geese was larger outside of migration and peak T_b occurred between 16:13 h (goose E, median time) and 07:05 h (goose D; Fig. S4 and Table S1). Furthermore, the daily range in T_b within geese was also larger outside of migration (mean±s.d. range across geese during migration: 2.49±0.35°C day^{-1}, outside of migration: 2.64±0.3°C day^{-1}, GLMM, Tukey's *post hoc* pairwise comparison, $P \leq 0.001$), with variation between geese explaining 19.06% of the

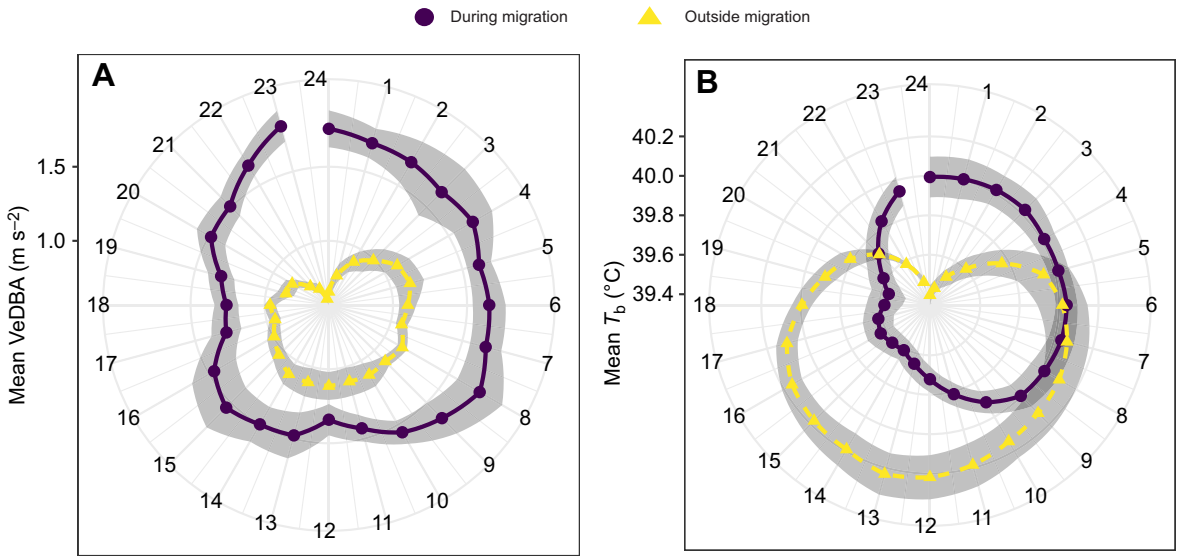

Fig. 2. Daily patterns of bar-headed goose T_b and activity. Circular plot showing daily patterns of mean activity and temperature. Mean (±s.e. as shaded ribbon around the mean) hourly (A) VeDBA and (B) T_b during migration and outside of migration (N=7 geese); for T_b plots per individual, see Fig. S4. The centre of the circles represents the lowest value of the *y*-axis and the concentric grey circles represent increasing values on the *y*-axis. Data were corrected to local time (hours on the spokes radiating from the centre).

Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

modelled variance. Goose G had the largest daily range in T_b both during (2.82°C day^{-1}) and outside of migration (3.03°C day^{-1}) and also displayed the greatest shift in T_b circadian rhythm during migration (Fig. S4).

Does T_b vary before, during and after migration?

We found that stage of year only explained 21.15% of variation in daily mean T_b. Accounting for temporal autocorrelation, mean daily T_b before migration (40.07°C, 39.86–40.29°C, 95% CI) was significantly higher than that during migration (39.76°C, 39.66–39.87°C, GLMM, Tukey's *post hoc* pairwise comparison, $P<0.001$). Mean daily T_b following migration was lower than that during migration (39.46°C, 39.02–39.9°C, GLMM, Tukey's *post hoc* pairwise comparison, $P=0.048$). Goose ID explained 45.74% of modelled variance. Goose G was found to be consistently warmer than the other geese across stages (Fig. 3).

Changes in T_b with flight effort and altitude

Does T_b change during flight?

We found that, in general, flight increased T_b relative to the start of flight; ΔT_b between the start and end of a flight across all geese was +0.39±0.16°C (grand median±s.d); however, there was a moderate amount of variation in the extent of this increase between geese (range 0.37°C in goose D to 0.71°C in goose C). Whilst T_b at the start was cooler than at the end of the flight (GLMM, −0.45°C, −0.38 to −0.51°C, 95% CI), this only explained 13.25% of the variance in T_b. Modelled variation between flights (comprising 38.96% of explained variance) was greater than that between geese (16.2%). Median±s.d. ΔT_b across all flights regardless of goose ID was 0.42±0.55°C (range −1.62 to 2.11°C), highlighting that T_b was reduced during some flights. Fig. 4 shows ΔT_b for each flight per goose. Increases in T_b were found to occur most rapidly at the beginning of flights (the maximum rate of T_b increase occurred within 10 min of the start of flight in 81% of flights lasting over 1 h; Fig. 1E). For example, in one typical flight completed by goose C in early December at overwintering grounds, T_b increased from 39.0 to 40.4°C in the first 8 min of flight, but then slowed to an overall mean±s.d. rate of change of 0.01±0.09°C min^{-1} and T_b was 41.3°C when the goose landed 154 min later (Fig. 5C).

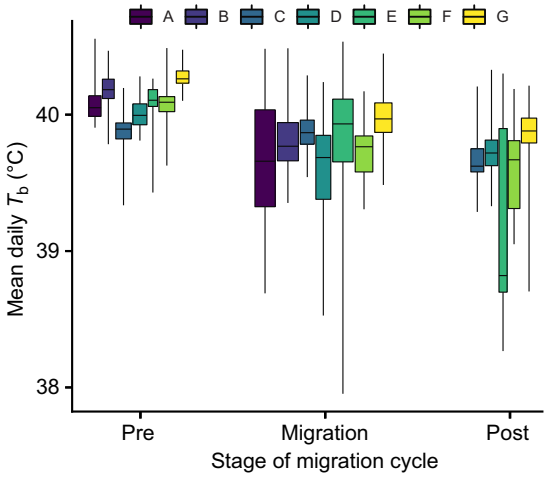

Fig. 3. Bar-headed goose T_b throughout the tracking period. Boxplots showing mean daily T_b at three stages of the tracking period relative to migration ($N=7$ geese pre-migration and during migration, $N=5$ post-migration). Boxes show interquartile range, horizontal black line inside the box shows median value, and whiskers show the minimum and maximum T_b per goose. Box width reflects the relative number of days per goose included in each case.

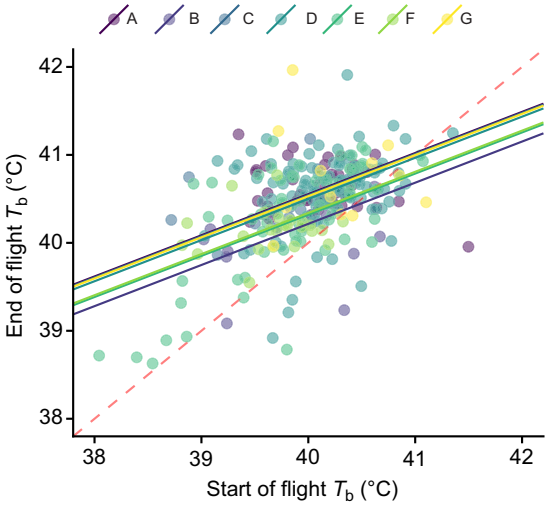

Fig. 4. Bar-headed goose T_b at the start and end of migratory flights. Dashed red line indicates line of equivalence [i.e. goose starts (*x*-axis) and finishes (*y*-axis) flight with the same T_b], points lying above the line of equivalence represent flights where the goose finished the flight warmer than it started, and points below the line indicate flights where the goose finished the flight cooler than it started. Data from all long flights (>60 min) were included. Individual geese ($N=7$) are shown in different colours.

In 50% of short flights (8–60 min, $n=917$ flights), flight stopped within 2 min of reaching the maximum T_b of that flight (mean maximum 40.5°C; Fig. 5D). This only occurred in 16 flights that lasted over 60 min (across the seven geese). Of these 16 flights, six were followed with cooling in the 10 min period immediately after the end of flight. Few similarities could be seen between these flights – they occurred between 1900 and 5000 m and were conducted by three geese (geese B, E and F). In the 10 min period after the flight, heat was lost at a mean rate of −0.03°C min^{-1} (−0.01 to −0.04°C min^{-1} range across geese). The maximum T_b reached at the end of these flights was 0.6°C higher (grand mean) than the mean T_b during all flights by each goose (39.94°C). In addition, there were cases when the geese continued to fly with a higher T_b than the peak T_b observed for the potentially interrupted flights; 91% of flights continued with a higher T_b than the T_b at which flight stopped for goose B, 20% for goose E and 29% for goose F (maximum T_b during flight anywhere was 1.65°C higher than the mean peak T_b across geese for stopped flights, range 1.03–2.83°C). The mean amount of time following a long flight before flying resumed was 83 min (range 6–1288 min) and 37.7% of long flights were followed by a period of at least 1 h before the geese flew again (Fig. 1E).

Does T_b change with altitude?

There was no clear effect of altitude on T_b, either for stationary or for flying geese (Fig. 6). The highest overall median±s.d. T_b (40.3±0.25°C) was found between 1000 and 2000 m and the lowest median T_b (39.7±0.6°C) was recorded between sea level and 1000 m (Fig. 6). *Post hoc* testing revealed that changes in T_b were variable across altitude bins. For example, between 4000 and 5000 m, a mean of 0.24°C heat was lost across geese, whilst between 5000 and 6000 m, 0.11°C heat was gained. At each altitude bin between 2000 and 5000 m, geese were progressively warmer during flight than when stationary (by 0.21, 0.47 and 0.63°C,

Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

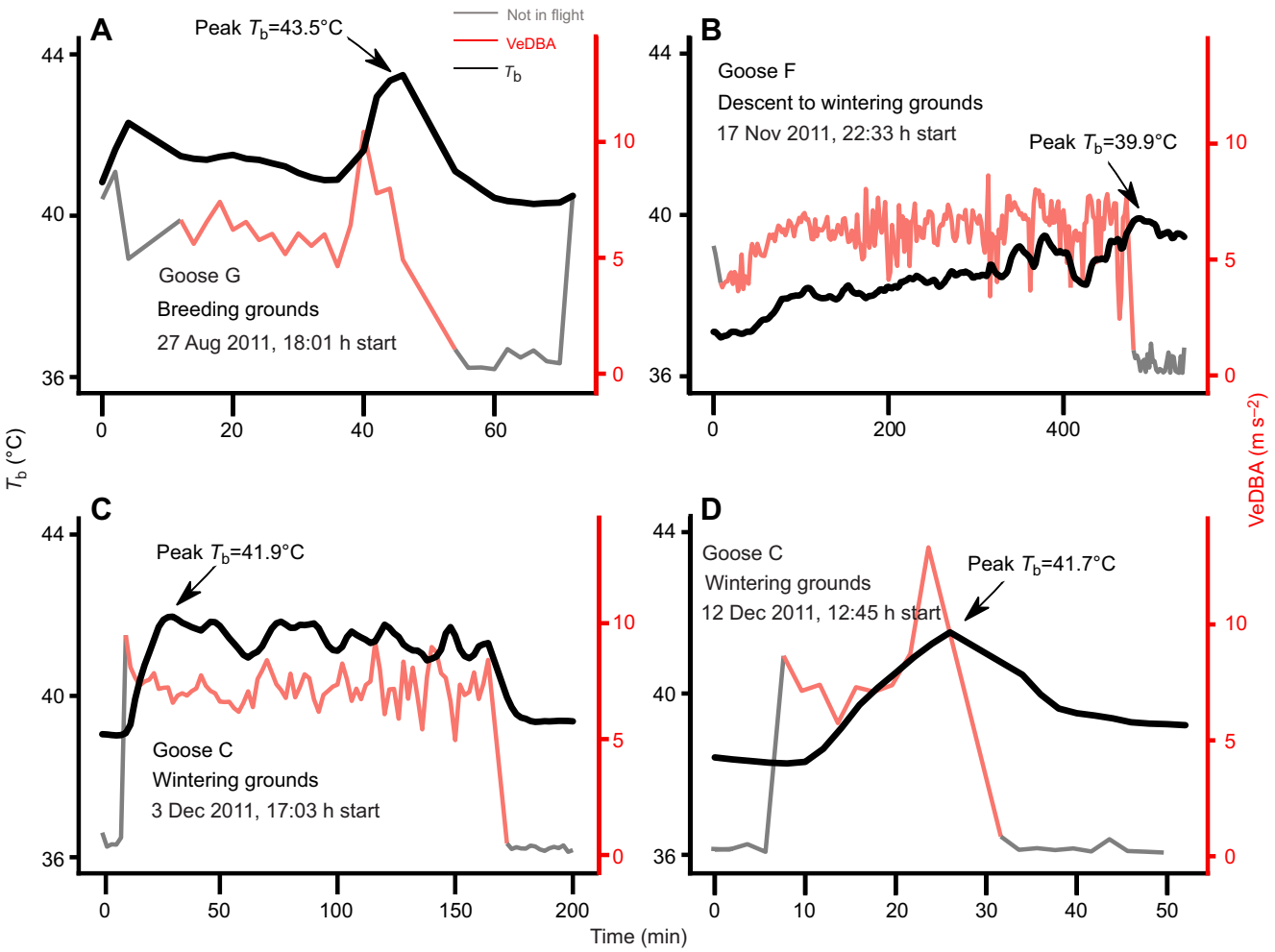

Fig. 5. Bar-headed goose T_b and activity profiles during four example flights. Flight T_b (black) and VeDBA profiles (red indicates flying periods, grey indicates non-flying periods before and after the individual flights). (A,D) Short flights, with rapid gains in T_b which may have caused the termination of flight. (B) A long flight with rises in T_b throughout the flight; flight stops at the hottest temperature logged during the flight. (C) Heat is gained rapidly at the start of flight, but a high rate of change of T_b is not sustained after the first 20 min of flight.

respectively, for altitude bins, Tukey's *post hoc* pairwise comparison, $P<0.001$). However, over 5000 m, T_b did not significantly differ according to flight status (i.e. it was the same for geese in flight and while stationary). It is of note that the effect of altitude on T_b whether flying or stationary was very small relative to the variation in T_b within each altitude bin (Fig. 6; Table S2), and these fixed effects explained only 3.56% of the variance in T_b, whereas individual flights or stationary bouts nested within geese accounted for 71.14% of explained variance.

Heart rate, rate of ascent/descent and altitude each affected T_b of individual geese differently and to varying extents (Fig. 7). Heart rate had the largest effect on T_b of all predictors, with a small positive trend in all geese, such that an increase of 100 beats min^{-1} caused a rise of 0.26°C (goose D) to 0.68°C (goose C; range of average effect across geese; Fig. 7A). However, the effect of heart rate on T_b was greatest at heart rates over 250 beats min^{-1}. T_b in geese C and G appeared to be the most sensitive to changes in heart rate, with increases in T_b between all adjacent heart rate bins over 250 beats min^{-1}. In contrast, goose D only gained temperature with increasing heart rate between 350–400 and 400–450 beats min^{-1}. Increases in altitude did not have a clear effect on T_b and goose T_b was very variable across altitude bins (Fig. 7C). Goose C responded the most to changes in altitude and significantly lowered T_b at each increase in altitude bin with the exception of between 4000 and 5000 m, where T_b increased by 0.66°C, and as a result was 0.57°C cooler over 6000 m than below 2000 m. However, the other geese were more variable in their response. Goose G was the least affected by altitude and maintained a consistent T_b between the lowest (1000–2000 m) and highest altitude bins (5000–6000 m). Rate of ascent had a very variable effect on T_b across geese (Fig. 7B). Goose D showed a strong relationship between rate of ascent and T_b, with faster climb rates causing the largest gains in T_b. In contrast, T_b in geese C and G was not affected by progressive increases in rate of ascent and had much wider 95% CI than in the other geese, suggesting more variation between flights. In addition to the high degree of variation between geese, there was also a high amount of variation between flights within geese, with flight ID as a random factor comprising 51.37% of the explained variance (mean value across geese; range: 42.11–69.08%), represented by the large overlapping confidence intervals shown in Fig. 7.

DISCUSSION

Overall, we found that despite the cold and inhospitable conditions of the Himalayas, bar-headed geese can maintain core body temperature within approximately 4°C, much like other birds during natural flight, regardless of the ambient conditions they

Journal of Experimental Biology (2019) 222, jeb203695. doi:10.1242/jeb.203695

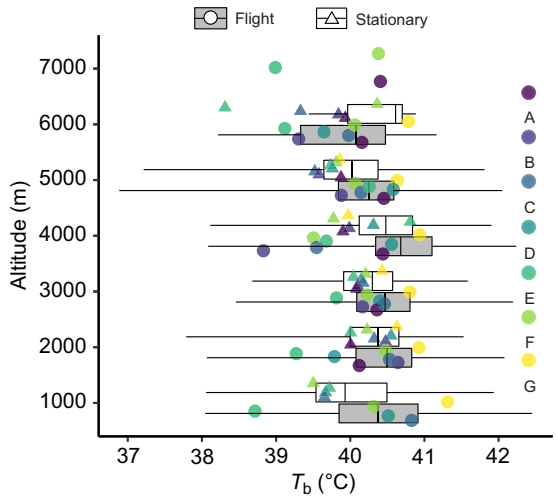

Fig. 6. Variation in bar-headed goose T_b during flight and whilst stationary at different altitudes. Boxes show interquartile range, median is shown by the vertical black line inside the box and whiskers show the minimum and maximum T_b across all geese. Coloured circles show median T_b for each goose whilst in flight and coloured triangles show median T_b for each goose whilst stationary (N=7).

experience or the altitude at which they fly. Therefore, we found little evidence that they find the cold conditions at high altitude limiting, or that they generate heat to intolerable excess when flying in warm temperatures (29–36°C) at their wintering grounds in India. We present evidence for a shift in circadian rhythm in response to migration such that bar-headed geese reach peak temperature early in the morning during migration, whereas outside of migration, peak T_b occurs during the daylight hours. We also found a smaller range in daily T_b during migration compared with before or after migration. Contrary to our predictions, observed changes in T_b were not driven by changes in altitude.

Does migration alter diurnal behaviour?

The earlier daily peak in T_b and activity during migration, even on days with no migratory flights, is a common response in migratory species (Gwinner, 1996; Rani et al., 2006) that persists even in captive birds (Zúñiga et al., 2016). This may confer additional advantages when migrating at high altitudes. Firstly, by flying at night when air temperatures are lower, birds can reduce flight speed (and associated metabolic costs) because cold air is denser. The increase in barometric pressure in cold conditions also leads to a higher partial pressure of oxygen, fuelling metabolism. In addition, by flying at night, bar-headed geese experience lower, more favourable wind speeds (Hawkes et al., 2012). Therefore, flight costs are minimised, and oxygen availability is maximised. Furthermore, during migration in song birds, it has been shown that metabolic costs incurred through thermoregulation during long stopover periods can exceed flight costs during migration (Hedenström and Alerstam, 1997, 1998) and that these costs are greater in cooler conditions (Wikelski et al., 2003), i.e. overnight. Stopover costs are also important during migration for waterfowl, particularly when environmental conditions can increase thermoregulatory requirements (O'Neal et al., 2018). When energy demand is high, waterfowl may feed both day and night in order to replenish fat stores (Nilsson, 1970; Mcneill et al., 1992) and in addition to this activity-induced thermogenesis, heat production is increased following food consumption (Weller, 1988). Therefore, the results of the present study suggest that, by increasing activity at night, bar-headed geese could mitigate both flight and thermoregulatory metabolic costs.

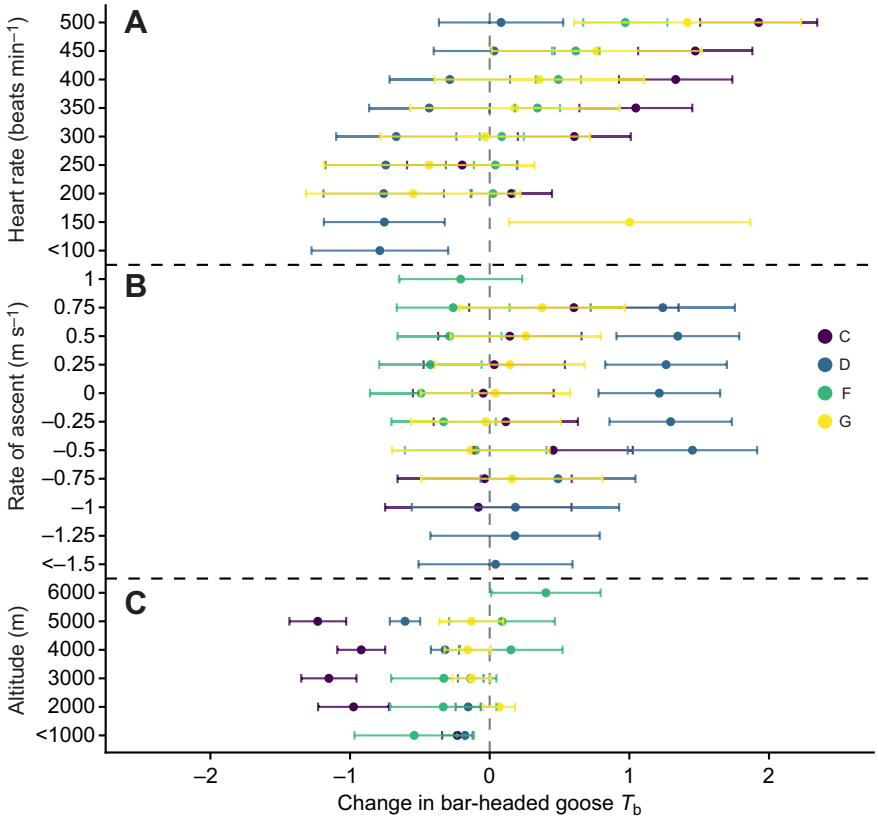

Fig. 7. The effect of altitude, heart rate and climb speed on flying bar-headed goose T_b. Dashed grey vertical line at 0 represents no effect of each predictor on T_b, and larger effect sizes (°C changes from mean T_b) in either direction indicate a stronger negative or positive effect of the predictor, for (A) heart rate, (B) rate of ascent and (C) altitude. Coloured circles show the mean effect per goose (N=4), and horizontal error bars represent the 95% CI. For example, at 2000 m altitude, goose C T_b dropped by 1°C on average. Altitude is grouped into 1000 m bins, rate of ascent/descent is grouped into 0.25 m s^{-1} bins, and heart rate is grouped into 50 beats min^{-1} bins. Data were filtered to include only long (over 1 h) flights and modelled using mixed models with flight ID as a random factor and temporal autocorrelation as accounted for using a corAR1 term.

Are there seasonal patterns in T_b?

Relative to T_b at breeding grounds, bar-headed geese were found to be approximately 0.3°C cooler during stationary periods of migration, resting between flights. This slight decrease in T_b occurs at sites on the Tibetan Plateau and Himalayan mountains at altitudes of ∼4500 m during the onset of autumn, when ambient temperatures are cold, so a cooler T_b during this period may help to reduce energy costs. However, the extent of this cooling is much smaller than might be predicted given that over the course of migration bar-headed goose T_b may vary by 3.8–5.8°C. Variation in T_b on this scale is not unusual in waterfowl. Greylag geese (*Anser anser*) show a yearly T_b range of more than 4°C, with peak T_b occurring in the summer months (probably due to both nesting in females and inter-individual interactions in males; Wascher et al., 2018). Furthermore, barnacle geese decrease T_b by 4.4°C at the start of migration over a 20 day period (Butler and Woakes, 2001). This was suggested to reduce energetic costs as thermoregulatory demands are lower, thus minimising fat depletion. Therefore, in the context of the present study, a drop of 0.3°C may have little effect on energy conservation of bar-headed geese. This suggests that bar-headed geese are able to both fuel flight and maintain thermal neutrality during migration. Furthermore, the bar-headed geese in this study were found to be warmest at breeding grounds and coolest at wintering grounds, despite the warmer ambient environments there. Therefore, reductions in T_b were not limited to the migratory period and are unlikely to be useful in energy conservation. Given the increased flight costs at high altitude, it is possible that a sustained T_b (and thus no compromise to flight muscle power; Carr and Lima, 2013) throughout migration may confer a greater advantage for flight in high-altitude environments than a reduction in T_b (and the potential for associated energy conservation).

Does T_b change with flight effort?

Bar-headed geese often gain heat through flight activity, though this was very variable across geese and flights, and could not be reliably predicted by changes in heart rate and rate of ascent. This suggests that the ecological context in which flights took place may be important. For example, on rare occasions, bar-headed geese can gain assistance from favourable winds (Bishop et al., 2015), which may result in fast rates of climb with little increase in metabolic effort. Other biotic factors, such as the use of formation flight to reduce costs (Portugal et al., 2014; Weimerskirch et al., 2001) may further modulate flight effort and heat gain during flight. If each goose completed a flight in the exact same location and at the same time of day, there might be much closer correlations between each flight, but in the natural world this may rarely be the case, highlighting the value of studying animals within the context of their own ever-changing environments.

With few exceptions we did not find evidence of excessive heat gain stopping flights by bar-headed geese, even when T_b of geese increased by 2.1°C during flight. In the few examples when flights did appear to stop at a peak T_b, the same geese flew continuously at other times with a higher T_b, suggesting that these temperatures were not the reason for the flight ending. This suggests that bar-headed geese do not experience an excess thermal burden through activity thermogenesis (even in the most extreme examples). This is contrary to findings in eider ducks and pigeons, in which heat gain through flight may constrain flight duration (Guillemette et al., 2016; Butler et al., 1977). However, hyperthermia in pigeons may not be directly comparable with that of wild migrating birds, as it was recorded in wind tunnels where flight durations are often shorter than in free-flying birds (Bishop et al., 2002). Furthermore, eider ducks, which are a diving species, may be more susceptible to hyperthermia than bar-headed geese not only because of their diving behaviour but also because of their high body weight to wing area ratio that increases flight costs (Guillemette et al., 2017). Eider ducks migrate in a series of short flights across bodies of ocean where they can land and offload heat via increased blood flow to the legs in the water (Kilgore and Schmidt-Nielsen, 1975), and while resting are protected by effective down insulation. In contrast, the lower wing loading typical of long-distance migratory waterfowl, including bar-headed geese (Lee et al., 2008), reduces flight costs and makes them better able to minimise heat gain during bouts of intense flapping flight, or when they arrive at overwintering grounds and experience warm ambient temperatures.

The rate of change of T_b was consistently highest immediately following the start of flight, suggesting that take-off and climbing lead to some heat gain. This heat gained in the first 10 min of flight is then attenuated throughout the flight, which could suggest that bar-headed geese make use of metabolic heat to increase T_b in a facultative way to enhance flight performance through an increase in reaction rate (Carr and Lima, 2013). T_b is then re-established at later phases of long flights, perhaps passively as the gradient between ambient temperature and T_b in bar-headed geese increases. Bar-headed geese may also be able to vasodilate their leg and beak vasculature during later phases of flight to offload heat to the environment (Midtgård, 1981; Scott et al., 2008; Tattersall et al., 2016).

We have described considerable variation in T_b in bar-headed geese, which varies more between geese and individual flights than with variation in heart rate, rate of ascent/descent or altitude. This may result in part from the small sample size of the present study, and it is possible that a larger sample size may reveal stronger correlations between heart rate and rates of ascent than in the present study. Goose G in particular maintained a stable T_b regardless of rate of ascent but displayed large variation between flights. It was also the largest goose in the study, and only completed a partial migration, in which it flew for shorter durations than the other geese. This highlights that in addition to the environmental factors probably impacting goose T_b, such as weather and specific flight costs, the individual physiology of each goose may modulate their T_b response to changing stimuli.

Does T_b change with altitude?

We found that bar-headed goose T_b does not appear to be reduced at high altitude. Given flight muscle is also a major thermogenic site in birds (Bicudo et al., 2002), it is likely that many adaptations to tolerate cold conditions are common to those meeting the demands of flight. Red knots (*Calidris canutus*) experimentally exposed to cold conditions were found to display adaptive changes relative to knots kept in a thermoneutral zone that mirror the physiological adjustments of species preparing to migrate; these include an increased body mass, heart mass and flight muscle mass as well as adjustments in enzyme activity including higher lipid metabolism and greater oxidative capacity (Vézina et al., 2017). Therefore, the adaptations that enable bar-headed geese to meet the high metabolic demands of flight whilst in hypoxia (Scott et al., 2015) may result in sufficient heat generation by the exercising muscle to maintain T_b even in the cold environments at high altitude. This was demonstrated in an earlier study that revealed captive bar-headed geese do not reduce their metabolic rate through reductions in T_b while at rest in severe hypoxia (Scott et al., 2008).

Bar-headed geese have an enhanced thermal sensitivity for haemoglobin–oxygen binding (Meir and Milsom, 2013), and it has been suggested that this trait could improve circulatory O_2 transport at cold ambient temperature. In the present study, we measured core (abdominal) T_b, and show that bar-headed geese generally maintained a consistent T_b regardless of altitude, which does not offer general support for this hypothesis. The continuing miniaturisation of biologging devices means the extent of regional heterothermy in large birds migrating across broad ranges of climatic conditions can now be discovered by simultaneously recording temperature at several locations of the body (Nord and Folkow, 2018). When this level of detail is combined with rich environmental data of greater spatial and temporal resolution than is possible at present, it may create a powerful tool revealing the extent and strategies of thermoregulation in migrating animals across taxa (McCafferty et al., 2015).

Acknowledgements
Work in Mongolia was conducted with permission from the Wildlife Science and Conservation Centre. We are grateful to the support of all the field team members in Mongolia, to A. Davies for developing the first generation of heart rate data loggers, to Robin Spivey for developing the logger that we used and the script to interpret the data, and to the work of Beaumaris Instruments Ltd in the development of housing for the instruments. We thank two anonymous reviewers for comments that improved the manuscript.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: C.M.B., N.B., P.J.B., W.K.M., G.R.S., L.A.H.; Methodology: N.P., C.M.B., N.B., P.J.B., B.C., W.K.M., G.R.S., L.A.H.; Software: N.P.; Formal analysis: N.P.; Investigation: N.P., C.M.B., L.A.H.; Resources: C.M.B., N.B.; Data curation: N.P., C.M.B.; Writing - original draft: N.P.; Writing - review & editing: N.P., C.M.B., P.J.B., W.K.M., G.R.S., L.A.H.; Visualization: N.P.; Supervision: L.A.H.; Project administration: C.M.B., N.B., P.J.B., B.C., W.K.M., G.R.S., L.A.H.; Funding acquisition: C.M.B., N.B., P.J.B., W.K.M., G.R.S., L.A.H.

Funding
Primary funding was from a UK Biotechnology and Biological Sciences Research Council (BBSRC) award to C.M.B. and P.J.B. (grant no. BB/FO15615/1) and a Natural Sciences and Engineering Research Council of Canada award to W.K.M., with additional support from the Max Planck Institute for Ornithology, the U.S. Geological Survey, Western Ecological and Patuxent Wildlife Research Centers, Avian Influenza Programme, and the Food and Agriculture Organization (FAO) through the Animal Health Service EMPRES surveillance program.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.203695.supplemental

References
Adamík, P., Emmenegger, T., Briedis, M., Gustafsson, L., Henshaw, I., Krist, M., Laaksonen, T., Liechti, F., Procházka, P., Salewski, V. et al. (2016). Barrier crossing in small avian migrants: individual tracking reveals prolonged nocturnal flights into the day as a common migratory strategy. *Sci. Rep.* **6**, 21560. doi:10.1038/srep21560

Altshuler, D. and Dudley, R. (2006). The physiology and biomechanics of avian flight at high altitude. *Integr. Comp. Biol.* **46**, 62-71. doi:10.1093/icb/icj008

Bernstein, M. H. and Duran, H. L. (1984). Extrapulmonary gas exchange enhances brain oxygen in pigeons. *Science* **226**, 564-566. doi:10.1126/science.6436975

Bevan, R. M., Boyd, I. L., Butler, P. J., Reid, K., Woakes, A. J. and Croxall, J. P. (1997). Heart rate and abdominal temperatures of free-ranging South Georgian shags, *Phalacrocorax georgianus*. *J. Exp. Biol.* **200**, 661-675.

Bicudo, J. E., Bianco, A. C. and Vianna, C. R. (2002). Adaptive thermogenesis in hummingbirds. *J. Exp. Biol.* **205**, 2267-2273.

Bishop, C. M., Ward, S., Woakes, A. J. and Butler, P. J. (2002). The energetics of barnacle geese (*Branta leucopsis*) flying in wild and captive conditions. *Comp. Biochem. Physiol.* **133**, 225-237. doi:10.1016/S1095-6433(02)00157-5

Bishop, C. M., Hawkes, L. A., Chua, B., Frappell, P. B., Milsom, W. K., Natsagdorj, T., Newman, S. H., Scott, G. R., Takekawa, J. Y., Wikelski, M. et al. (2015). The roller coaster flight strategy of bar-headed geese conserves energy during Himalayan migrations. *Science* **347**, 250-254. doi:10.1126/science.1258732

Brodsky, L. M. and Weatherhead, P. J. (1984). Behavioural thermoregulation in wintering black ducks: roosting and resting. *Can. J. Zool.* **62**, 1223-1226. doi:10.1139/z84-177

Butler, P. J. (2010). High fliers: the physiology of bar-headed geese. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **156**, 325-329. doi:10.1016/j.cbpa.2010.01.016

Butler, P. J. and Woakes, A. J. (2001). Seasonal hypothermia in a large migrating bird: saving energy for fat deposition? *J. Exp. Biol.* **204**, 1361-1367.

Butler, P. J., West, N. H. and Jones, D. R. (1977). Respiratory and cardiovascular responses of the pigeon to sustained, level flight in a wind-tunnel. *J. Exp. Biol.* **71**, 7-26.

Carpenter, F. L. and Hixon, M. A. (1988). A new function for torpor: fat conservation in a wild migrant hummingbird. *Condor* **90**, 373-378. doi:10.2307/1368565

Carr, J. M. and Lima, S. L. (2013). Nocturnal hypothermia impairs flight ability in birds: a cost of being cool. *Proc. R. Soc. B Biol. Sci.* **280**. doi:10.1098/rspb.2013.1846

Ciancio, J. E., Quintana, F., Sala, J. E. and Wilson, R. P. (2016). Cold birds under pressure: can thermal substitution ease heat loss in diving penguins? *Mar. Biol.* **163**, 1-15. doi:10.1007/s00227-016-2813-2

Clarke, A. and Rothery, P. (2008). Scaling of body temperature in mammals and birds. *Funct. Ecol.* **22**, 58-67.

Dawson, W. R. (1982). Evaporative losses of water by birds. *Comp. Biochem. Physiol. A Physiol.* **71**, 495-509. doi:10.1016/0300-9629(82)90198-0

Dawson, W. R. and Carey, C. (1976). Seasonal acclimatization to temperature in cardueline finches. *J. Comp. Physiol.* **112**, 317-333. doi:10.1007/BF00692302

Ding, J., Cuo, L., Zhang, Y. and Zhu, F. (2018). Monthly and annual temperature extremes and their changes on the Tibetan Plateau and its surroundings during 1963–2015. *Sci. Rep.* **8**, 1-23. doi:10.1038/s41598-018-30320-0

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* **66**, 239-274. doi:10.1146/annurev.physiol.66.032102.115105

Guillemette, M., Woakes, A. J., Larochelle, J., Polymeropoulos, E. T., Granbois, J.-M., Butler, P. J., Pelletier, D., Frappell, P. B. and Portugal, S. J. (2016). Does hyperthermia constrain flight duration in a short-distance migrant? *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150386. doi:10.1098/rstb.2015.0386

Guillemette, M., Polymeropoulos, E. T., Portugal, S. J. and Pelletier, D. (2017). It takes time to be cool: on the relationship between hyperthermia and body cooling in a migrating seaduck. *Front. Physiol.* **8**, 532. doi:10.3389/fphys.2017.00532

Gwinner, E. (1996). Circadian and circannual programmes in avian migration. *J. Exp. Biol.* **199**, 39-48.

Hahn, S., Bauer, S. and Liechti, F. (2009). The natural link between Europe and Africa - 2.1 billion birds on migration. *Oikos* **118**, 624-626. doi:10.1111/j.1600-0706.2008.17309.x

Harrison, X. A., Donaldson, L., Eugenia Correa-Cano, M., Evans, J., Fisher, D. N., Goodwin, C., Robinson, B., Hodgson, D. J., Inger, R., Author, C. et al. (2017). Best practice in mixed effects modelling and multi-model inference in ecology. *PeerJ* **6**, e3113v1. doi:10.7287/peerj.preprints.3113v1

Hawkes, L. A., Balachandran, S., Batbayar, N., Butler, P. J., Frappell, P. B., Milsom, W. K., Tseveenmyadag, N., Newman, S. H., Scott, G. R., Sathiyaselvam, P. et al. (2011). The trans-Himalayan flights of bar-headed geese (*Anser indicus*). *Proc. Natl. Acad. Sci. USA* **108**, 9516-9519. doi:10.1073/pnas.1017295108

Hawkes, L. A., Balachandran, S., Batbayar, N., Butler, P. J., Chua, B., Douglas, D. C., Frappell, P. B., Hou, Y., Milsom, W. K., Newman, S. H. et al. (2012). The paradox of extreme high-altitude migration in bar-headed geese *Anser indicus*. *Proc. R. Soc. B* **280**, 20122114. doi:10.1098/rspb.2012.2114

Hedenström, A. and Alerstam, T. (1997). Optimum fuel loads in migratory birds: distinguishing between time and energy minimization. *J. Theor. Biol.* **189**, 227-234. doi:10.1006/jtbi.1997.0505

Hedenström, A. and Alerstam, T. (1998). How fast can birds migrate? *J. Avian Biol.* **29**, 424-432. doi:10.2307/3677161

Hiebert, S. M. (1990). Energy costs and temporal organization of torpor in the rufous hummingbird (*Selasphorus rufus*). *Div. Comp. Physiol. Biochem. Soc. Integr. Comp. Biol.* **63**, 1082-1097. doi:10.1086/physzool.63.6.30152634

Hiebert, S. M. and Noveral, J. (2007). Are chicken embryos endotherms or ectotherms? A laboratory exercise integrating concepts in thermoregulation and metabolism. *Adv. Physiol. Educ.* **31**, 97-109. doi:10.1152/advan.00035.2006

Hu, G., Lim, K. S., Horvitz, N., Clark, S. J., Reynolds, D. R., Sapir, N. and Chapman, J. W. (2016). Mass seasonal bioflows of high-flying insect migrants. *Science* **354**, 420-424. doi:10.1126/science.aah4379

Jammalamadaka, S. R. and Lund, U. J. (2006). The effect of wind direction on ozone levels: a case study. *Environ. Ecol. Stat.* **13**, 287-298. doi:10.1007/s10651-004-0012-7

Kenward, R. E. (2000). *A Manual for Wildlife Radio Tagging*. Academic Press.

Kilgore, J. D. L. and Schmidt-Nielsen, K. (1975). Heat loss from ducks' feet immersed in cold water. *Condor* **77**, 475-478. doi:10.2307/1366094

Lee, S. Y., Scott, G. R. and Milsom, W. K. (2008). Have wing morphology or flight kinematics evolved for extreme high altitude migration in the bar-headed goose? *Comp. Biochem. Physiol C Toxicol. Pharmacol.* **148**, 324-331. doi:10.1016/j.cbpc.2008.05.009

McCafferty, D. J., Gallon, S. and Nord, A. (2015). Challenges of measuring body temperatures of free-ranging birds and mammals. *Anim. Biotelemetry* **3**, 1-10. doi:10.1186/s40317-015-0075-2

McKechnie, A. E. and Lovegrove, B. G. (2002). Avian facultative hypothermic responses: a review. *Condor* **104**, 705-724. doi:10.1093/condor/104.4.705

McKechnie, A. E. and Wolf, B. O. (2019). The physiology of heat tolerance in small endotherms. *Physiology* **34**, 302-313. doi:10.1152/physiol.00011.2019

McKinney, R. and McWilliams, S. (2005). A new model to estimate daily energy expenditure for wintering waterfowl. *Wilson Bull.* **117**, 44-55. doi:10.1676/04-060

McNeill, B. Y. R., Drapeau, P. and Goss-custard, J. D. (1992). The occurrence and adaptive significance of nocturnal habits in waterfowl. *Biol. Rev.* **67**, 381-419. doi:10.1111/j.1469-185X.1992.tb01188.x

Meir, J. U. and Milsom, W. K. (2013). High thermal sensitivity of blood enhances oxygen delivery in the high-flying bar-headed goose. *J. Exp. Biol.* **216**, 2172-2175. doi:10.1242/jeb.085282

Midtgård, U. (1981). The Rete tibiotarsale and Arterio-venous association in the hind limb of birds: a comparative morphological study on counter-current heat exchange systems. *Acta Zool.* **62**, 67-87. doi:10.1111/j.1463-6395.1981.tb00617.x

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133-142. doi:10.1111/j.2041-210x.2012.00261.x

Nilsson, L. (1970). Food-seeking activity of south swedish diving ducks in the non-breeding season. *Nord. Soc. Oikos1* **21**, 145-154. doi:10.2307/3543670

Nord, A. and Folkow, L. P. (2018). Seasonal variation in the thermal responses to changing environmental temperature in the world's northernmost land bird. *J. Exp. Biol.* **221**. doi:10.1242/jeb.171124

O'Neal, B. J., Stafford, J. D., Larkin, R. P. and Michel, E. S. (2018). The effect of weather on the decision to migrate from stopover sites by autumn-migrating ducks. *Mov. Ecol.* **6**, 1-9. doi:10.2307/3543670

Pennycuick, C. J. (1969). The mechanics of bird migration. *Ibis (Lond. 1859)* **111**, 525-556. doi:10.1111/j.1474-919X.1969.tb02566.x

Pinshow, B., Bernstein, M. H. and Arad, Z. (1985). Effects of temperature and PCO_2 on O_2 affinity of pigeon blood: implications for brain O_2 supply. *Am. J. Physiol.* **249**, R758-R764. doi:10.1152/ajpregu.1985.249.6.R758

Portugal, S. J., Hubel, T. Y., Fritz, J., Heese, S., Trobe, D., Voelkl, B., Hailes, S., Wilson, A. M. and Usherwood, J. R. (2014). Upwash exploitation and downwash avoidance by flap phasing in ibis formation flight. *Nature* **505**, 399-402. doi:10.1038/nature12939

Qasem, L., Cardew, A., Wilson, A., Griffiths, I., Halsey, L. G., Shepard, E. L. C., Gleiss, A. C. and Wilson, R. (2012). Tri-axial dynamic acceleration as a proxy for animal energy expenditure; should we be summing values or calculating the vector? *PLoS ONE* **7**. doi:10.1371/journal.pone.0031187

Rani, S., Malik, S., Trivedi, A. K., Singh, S. and Kumar, V. (2006). A circadian clock regulates migratory restlessness in the blackheaded bunting, *Emberiza melanocephala*. *Curr. Sci.* **91**, 1093-1096. doi:10.3109/07420528.2011.635234

Scott, G. R., Cadena, V., Tattersall, G. J. and Milsom, W. K. (2008). Body temperature depression and peripheral heat loss accompany the metabolic and ventilatory responses to hypoxia in low and high altitude birds. *J. Exp. Biol.* **211**, 1326-1335. doi:10.1242/jeb.015958

Scott, G. R., Hawkes, L. A., Frappell, P. B., Butler, P. J., Bishop, C. M. and Milsom, W. K. (2015). How bar-headed geese fly over the himalayas. *Physiology* **30**, 107-115. doi:10.1152/physiol.00050.2014

Somveille, M., Rodrigues, A. S. L. and Manica, A. (2015). Why do birds migrate? A macroecological perspective. *Glob. Ecol. Biogeogr.* **24**, 664-674. doi:10.1111/geb.12298

Spivey, R. J. and Bishop, C. M. (2014). An implantable instrument for studying the long-term flight biology of migratory birds. *Rev. Sci. Instrum.* **85**. doi:10.1063/1.4854635

Tattersall, G. J., Arnaout, B. and Symonds, M. R. E. (2016). The evolution of the avian bill as a thermoregulatory organ. *Biol. Rev.* **92**, 1630-1656. doi:10.1111/brv.12299

Tombre, I. M., Høgda, K. A., Madsen, J., Griffin, L. R., Kuijken, E., Shimmings, P., Rees, E. and Verscheure, C. (2008). The onset of spring and timing of migration in two arctic nesting goose populations: The pink-footed goose *Anser brachyrhynchus* and the barnacle goose *Branta leucopsis*. *J. Avian Biol.* **39**, 691-703. doi:10.1111/j.1600-048X.2008.04440.x

Vézina, F., Gerson, A. R., Guglielmo, C. G. and Piersma, T. (2017). The performing animal: causes and consequences of body remodeling and metabolic adjustments in red knots facing contrasting thermal environments. *Am. J. Physiol. Integr. Comp. Physiol.* **313**, R120-R131. doi:10.1152/ajpregu.00453.2016

Wascher, C. A. F., Kotrschal, K. and Arnold, W. (2018). Free-living greylag geese adjust their heart rates and body core temperatures to season and reproductive context. *Sci. Rep.* **8**, 1-8. doi:10.1038/s41598-017-17765-5

Weimerskirch, H., Martin, J., Clerquin, Y., Alexandre, P. and Jiraskova, S. (2001). Energy saving in flight formation. *Nature* **413**, 697-698. doi:10.1038/35099670

Weller, M. W. (1988). *Waterfowl in Winter: Selected Papers from Symposium and Workshop Held in Galveston, Texas, 7-10 January 1985*. (ed. M. W. Weller). University of Minnesota Press.

Welton, N. J., Houston, A. I., Ekman, J. and McNamara, J. M. (2002). A dynamic model of hypothermia as an adaptive response by small birds to winter conditions. *Acta Biotheor.* **50**, 39-56. doi:10.1023/A:1014761227478

Whitworth, D., Newman, S., Mundkur, T. and Harris, P. (2007). Wild bird capture techniques. In *Wild Birds and Avian Influenza. An Introduction to Applied Field Research and Disease Sampling Techniques*, FAO Animal Production and Health Manual No. 5, pp. 33-50.

Wikelski, M., Tarlow, E. M., Raim, A., Diehl, R. H., Larkin, R. P. and Visser, G. H. (2003). Costs of migration in free-flying songbirds. *Nature* **423**, 704. doi:10.1038/423704a

Williams, C. K., Dugger, B. D., Brasher, M. G., Coluccy, J. M., Cramer, D. M., Eadie, J. M., Gray, M. J., Hagy, H. M., Livolsi, M., Mcwilliams, S. R. et al. (2014). Estimating habitat carrying capacity for migrating and wintering waterfowl: considerations, pitfalls and improvements. *Wildfowl* 407-435.

Williams, C. L., Sato, K. and Ponganis, P. J. (2019). Activity not submergence explains diving heart rates of captive loggerhead turtles. *J. Exp. Biol.* jeb.200824. doi:10.1242/jeb.200824

Withers, P. C. (1977). Respiration, metabolism, and heat exchange of euthermic and torpid poorwills and hummingbirds. *Physiol. Zool.* **50**, 43-52. doi:10.1086/physzool.50.1.30155714

Zúñiga, D., Falconer, J., Fudickar, A. M., Jensen, W., Schmidt, A., Wikelski, M. and Partecke, J. (2016). Abrupt switch to migratory night flight in a wild migratory songbird. *Sci. Rep.* **6**, 1-10. doi:10.1038/s41598-016-0001-8